=> d his (FILE 'HOME' ENTERED AT 19:34:48 ON 29 APR 2003) FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 19:35:00 ON 29 APR 2003 290545 S TREHALOSE OR OLIGOSACCHARIDE OR POLYSACCHARIDE L1 2077 S LOAD? (6A) PLATELET L221 S L1 AND L2 L3 9 DUP REM L3 (12 DUPLICATES REMOVED) L437298 S LOAD? (7A) (PLATELET OR CELL) L5 170 S L1 AND L5 L6 35190 S LOAD? (6A) (PLATELET OR CELL) L781 S L1(S)L7 L8 50 DUP REM L8 (31 DUPLICATES REMOVED) L9 => d au ti so ab 1-9 14 DUPLICATE 1 ANSWER 1 OF 9 MEDLINE L4Crowe John H; Tablin Fern; Wolkers Willem F; Gousset Karine; Tsvetkova ΑU Nelly M; Ricker Josette Stabilization of membranes in human platelets freeze-dried with ΤI CHEMISTRY AND PHYSICS OF LIPIDS, (2003 Jan) 122 (1-2) 41-52. SO Journal code: 0067206. ISSN: 0009-3084. Human blood platelets are normally stored in blood banks for 3-5 days, AB after which they are discarded. We have launched an effort at developing means for preserving the platelets for long term storage. In previous studies we have shown that trehalose can be used to preserve biological membranes and proteins during drying and have provided evidence concerning the mechanism. A myth has grown up about special properties of trehalose, which we discuss here and clarify some of what is fact and what is misconception. We have found a simple way of introducing this sugar into the cytoplasm of platelets and have successfully freeze-dried the trehalose-loaded platelets, with very promising results. We present evidence that membrane microdomains are

L4 ANSWER 2 OF 9 MEDLINE DUPLICATE 2

AU Wolkers Willem F; Looper Sheri A; McKiernan Ariane E; Tsvetkova Nelly M; Tablin Fern; Crowe John H

Finally, we propose a possible mechanism by which the microdomains are

maintained intact in the platelets freeze-dried with trehalose.

TI Membrane and protein properties of freeze-dried mouse platelets.

SO MOLECULAR MEMBRANE BIOLOGY, (2002 Jul-Sep) 19 (3) 201-10.

preserved.

AB

Journal code: 9430797. ISSN: 0968-7688. Membrane properties and the overall protein secondary structure of freeze-dried trehalose-loaded mouse platelets were studied using steady state fluorescence anisotropy and Fourier transform infrared spectroscopy (FTIR). FTIR results showed that fresh control mouse platelets have a main phase transition at approximately 14 degrees C, whereas, freeze-dried platelets exhibited a main phase transition approximately 12 degrees C. However, the cooperativity of the transition of the rehydrated platelets was greatly enhanced compared to that of control platelets. Anisotropy experiments performed with 1,6 diphenyl-1,3,5 hexatriene (DPH) complemented FTIR results and showed that the lipid order in the core of the membrane was affected by freeze-drying procedures. Similar experiments with trimethyl ammonium 1,6 diphenyl-1,3,5 hexatriene (TMA-DPH), a membrane surface probe, indicated that membrane properties at the membrane/water interface were less affected by freeze-drying procedures than the core of the membrane. Lyophilization did not result in massive protein denaturation, but the overall protein secondary structure was altered, based on in situ assessment of the amide-I and amide-II band profiles.

Lyophilization-induced changes to endogenous platelet proteins were further investigated by studying the protein's heat stability. In fresh control platelets, proteins denatured at 42 degrees C, whereas proteins in the rehydrated platelets denatured at 48 degrees C. ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS L4Wolkers, Willem F.; Crowe, John H.; Tablin, Fern; Oliver, Ann E.; Walker, IN Naomi J. Stabilization of therapeutic platelets ΤI PCT Int. Appl., 36 pp. SO CODEN: PIXXD2 A dehydrated compn. is provided that includes freeze-dried platelets. AB platelets are loaded with trehalose which preserves biol. properties during freeze-drying and rehydration. trehalose loading is conducted at temps. from 25 to 40.degree., most preferably at 37.degree., with the loading soln. having trehalose in an amt. from about 10 mM to about 50 mM. These freeze-dried platelets are substantially shelf-stable and are rehydratable so as to have a normal response to an agonist, e.g., thrombin, with virtually all of the platelets participating in clot formation within about 3 min at 37.degree.. Platelet suspensions were prepd. and ristocetin was added to the platelet. The clot formation was 95-100% for the agonist tested. ANSWER 4 OF 9 CAPLUS COPYRIGHT 2003 ACS L4Stienstra, Stoffer IN Platelet stabilization by treatment with carbohydrates TIPCT Int. Appl., 10 pp. SO CODEN: PIXXD2 Disclosed ia a method for the prodn. of stabilized platelets, comprises AB the steps of: (i) pre-activating platelets, to induce the formation of microvesicles; (ii) contacting the pre-activated platelets with a carbohydrate, esp. trehalose, whereby the carbohydrate is incorporated into the platelets; and (iii) drying the thusloaded platelets. ANSWER 5 OF 9 CAPLUS COPYRIGHT 2003 ACS L4Roser, Bruce J.; De Vos, Diana IN Compositions for for stabilizing platelets for dry storage ΤI U.S. Pat. Appl. Publ., 15 pp., Cont. of U.S. Ser. No. 366,810, abandoned. SO CODEN: USXXCO The invention provides methods for drying platelets to obtain compns. AB which are storage stable over a wide range of temps. and for an extended period of time. The invention also provides methods for permeabilizing platelets which allows them to be loaded with various compds. Platelets were acid permeabilized. After addn. of stop buffer, the mixt. was centrifuged at room temp. at 1800 rpm for 10 min to pellet the platelets. Drying buffer was prepd. by bringing the pH of HEPES-buffered saline to 7.0 using 2M and 0.2M NaOH. To 10 mL of this buffer 50 .mu.L hirudin (10 U/mL); 6.25 .mu.L apyrase (20 U/mL); 1 mg magnesium sulfate; 0.1 g trehalose; and 0.1 g. BSA were added. Resuspended platelets (300 .mu.L) was carefully pipetted into 3 mL siliconized glass pharmaceutical vials and dried. DUPLICATE 3 MEDLINE ANSWER 6 OF 9 L4Wolkers W F; Walker N J; Tablin F; Crowe J H ΑU Human platelets loaded with trehalose ΤI survive freeze-drying. CRYOBIOLOGY, (2001 Mar) 42 (2) 79-87. SO Journal code: 0006252. ISSN: 0011-2240. Human blood platelets are stored in blood banks for 5 days, after which AΒ they are discarded, by federal regulation. This short lifetime has led to a chronic shortage of platelets, a problem that is particularly acute in immunosuppressed patients, such as those with AIDS. We report here that

platelets can be preserved by freeze-drying them with trehalose, a sugar found at high concentrations in organisms that naturally survive drying. We suggest that these findings will obviate the storage problem with platelets. Trehalose is rapidly taken up by human platelets at 37 degrees C, with loading efficiencies of 50% or greater. Fluid-phase endocytosis plays an important role in this efficient uptake of trehalose, but other mechanisms may also be involved. Trehalose-loaded platelets were successfully freeze-dried, with excellent recovery of intact platelets. Rehydration from the vapor phase led to a survival rate of 85%. The response of these platelets to the agonists thrombin (1 U/ml), collagen (2 microg/ml), ADP (20 micromM), and ristocetin (1.6 mg/ml) was almost identical to that of fresh, control platelets. Analysis by Fourier transform infrared spectroscopy demonstrated that the membrane and protein components of trehalose-loaded platelets after freeze-drying, prehydration, and rehydration were remarkably similar to those of fresh platelets.

ANSWER 7 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. L4

Tablin, Fern (1); Wolkers, Willem F.; Walker, Naomi J. (1); Crowe, John H. ΑU

Trehalose loaded, freeze-dried human platelets ΤI are functional and retain normal protein structure and membrane phospholipid phase transitions.

Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 62a. print. SO Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology

. ISSN: 0006-4971.

Human platelets can be preserved by freeze-drying them with trehalose, a sugar found at high concentrations in organisms that naturally survive drying. Trehalose is rapidly taken up by human platelets at 37oC, with loading efficiencies of 50% or greater. Fluid phase endocytosis plays an important role in this remarkably efficient uptake of trehalose. Trehaloseloaded platelets were successfully freeze-dried with excellent recovery of intact platelets. Rehydration from the vapor phase led to a surival rate of 85% Aggregometry of rehydrated freeze-dried platelets demonstrated that they were responsive to thrombin (1U/ml) collagen (2mug/ml) ADP (20muM) and ristocetin (1.6mg/ml), in an almost identical manner to fresh control platelets. Analysis by Fourier transform infrared (FTIR) spectroscopy of the amide II region (1550 cm-1), demonstrated that the protein components of trehaloseloaded platelets after freeze-drying, prehydration and rehydration were remarkably similar to fresh platelets containing primarily beta sheet and turn structures. Treatment of fresh platelets with thrombin showed platelet denaturation as demonstrated by the presence of significant numbers of alpha helixes. Analysis of membrane phospholipid phase transitions by FTIR demonstrated that trehaloseloaded freeze-dried rehydrated platelets had a phase transition virtually identical to that of control fresh platelets. Thrombin activated platelets, by comparison, showed several transitions, suggestive of phase separation. Trehalose-loaded freeze-dried platelets are stable for up to 2 weeks at -20oC and remain stable once rehydrated for up to six hours. These studies demonstrate that we can successfully load, freeze-dry and rehydrate non-fixed platelets, and have them maintain normal structure and function in the rehydrated state.

ANSWER 8 OF 9 MEDLINE L4

DUPLICATE 4

Reid T J; Esteban G; Clear M; Gorogias M ΑU

Platelet membrane integrity during storage and activation. ΤI

TRANSFUSION, (1999 Jun) 39 (6) 616-24. SO Journal code: 0417360. ISSN: 0041-1132.

BACKGROUND: The platelet cell membrane appears to undergo a lipid-phase AB

transition on cooling from 23 degrees C to 4 degrees C. Consequences of this phase transition are leakage of cellular material and irreversible cellular damage. Whether agents, of known benefit in protecting membranes and proteins from cooling and drying injury, could also protect platelets was investigated. Leakage of cytosolic components was assessed by measuring the release of fluorescein into the surrounding medium. DESIGN AND METHODS: Fresh platelets were suspended in 5 percent dimethyl sulfoxide (DMSO) or in 5 mM of one the following agents: glucose, trehalose, sucrose, glycerol, ethylene glycol, 1,2-propanediol, or L-proline. Platelets were loaded with 10 nMfluorescein diacetate (FD), chilled at 4 degrees C for 24 hours or frozen at -1 degree C per minute to -70 degrees C, warmed rapidly at 37 degrees C, and centrifuged, and the supernatant was measured for the presence of fluorescein. The effect of FD on platelets was assessed by agglutination with ristocetin, aggregation with thrombin and ADP, platelet-induced clot retraction, and expression of p-selectin. Platelet function and activation before and after freezing or cooling were measured by the same methods. RESULTS: By flow cytometry, 98 percent of the platelets incorporated FD. The trapped fluorescein resulted in neither platelet activation (p = 0.9) nor reduction of platelet function (p =0.12-0.94) from that in control platelets. Freezing of platelets in DMSO caused far less release of fluorescein than did freezing with other agents (p<0.001) or chilling of platelets at 4 degrees C for 24 hours (p<0.0001). Supernatant levels of fluorescein correlated inversely with platelet function. Fluorescein was also shown to be released during aggregation with thrombin or ADP but not during agglutination with ristocetin. CONCLUSIONS: Release of fluorescein into the surrounding medium indicated a loss of platelet membrane integrity and function. Cellular loading with FD is a simple method of studying membrane integrity of platelets and other cells.

L4 ANSWER 9 OF 9 MEDLINE

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DUPLICATE 5

AU Hildreth J E; Derr D; Azorsa D O

TI Characterization of a novel self-associating Mr 40,000 platelet glycoprotein.

SO BLOOD, (1991 Jan 1) 77 (1) 121-32. Journal code: 7603509. ISSN: 0006-4971.

A novel platelet glycoprotein has been purified and characterized. This glycoprotein, designated Pltgp40, is an acidic sialylated 40,000-dalton protein that bears both O-linked and N-linked oligosaccharides. Treatment of Pltgp40 with neuraminidase resulted in a 5,000-dalton reduction in its Mr and a 1.5 Unit alkaline shift in the isoelectric point, indicating the presence of a large number of sialic acid residues. A similar size reduction and change in pl were observed after treatment of Pltgp40 with O-glycanase showing that sialic acids are present on O-linked oligosaccharides. Digestion of Pltgp40 with N-glycanase reduced the Mr to approximately 20,000 daltons but did not affect the isoelectric point, suggesting that Pltgp40 contains six to seven nonsialylated N-linked carbohydrate chains. High Mr proteins were observed in affinity purified Pltgp40 and were identified as detergent-stable protein oligomers consisting of multiple 40,000-dalton monomers. Immunodepletion and direct binding studies indicated that Pltgp40 was not equivalent to Ig Fc receptor type II, another 40,000-dalton glycoprotein expressed on platelets. However, Pltgp40 copurified with Fc receptor type II when platelet extracts were loaded onto human IgG affinity columns, raising the possibility that Pltgp40 may associate with Fc receptors or Fc receptor-lg complexes. Amino acid sequence analysis of the N-terminus of Pltgp40 was performed and confirmed that Pltgp40 is a novel platelet glycoprotein. Epitopes on Pltgp40 appear to be widely expressed because monoclonal antibodies against Pltgp40 also reacted with a variety of myeloid, lymphoid, and epithelial cells. Pltgp40 was detected on activated but not resting platelets, indicating that Pltgp40 is a platelet activation marker.

=> d bib 3-5 14 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS L42001:597749 CAPLUS AN135:170781 DN Stabilization of therapeutic platelets TI Wolkers, Willem F.; Crowe, John H.; Tablin, Fern; Oliver, Ann E.; Walker, IN The Regents of the University of California, USA PA PCT Int. Appl., 36 pp. so CODEN: PIXXD2 Patent DT LA English FAN.CNT 3 APPLICATION NO. DATE KIND DATE PATENT NO. _____ WO 2001-US4224 20010208 WO 2001058266 A1 20010816 PΙ W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG EP 2001-907169 20010208 A1 20021113 EP 1255439 AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR 20000210 PRAI US 2000-501773 Α 20010208 WO 2001-US4224 W THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD RE.CNT 1 ALL CITATIONS AVAILABLE IN THE RE FORMAT ANSWER 4 OF 9 CAPLUS COPYRIGHT 2003 ACS **T.4** 2001:319654 CAPLUS ΑN DN 134:331585 Platelet stabilization by treatment with carbohydrates TI Stienstra, Stoffer TN Quadrant Holdings Cambridge Limited, UK PA PCT Int. Appl., 10 pp. SO CODEN: PIXXD2 DT Patent LA English FAN.CNT 1 APPLICATION NO. DATE KIND DATE PATENT NO. ______ ----_____ 20001023 WO 2000-GB4078 WO 2001030141 20010503 A1 PI · W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG EP 2000-972970 20001023 EP 1221835 A1 20020717 20030409 EP 1221835 В1 AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL JP 2001-532581 20030402

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19991112 GB 1999-26838 Α 20000522 Α GB 2000-12372 20001023 WO 2000-GB4078 W THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT ANSWER 5 OF 9 CAPLUS COPYRIGHT 2003 ACS 2001:868949 CAPLUS 136:11285 Compositions for for stabilizing platelets for dry storage Roser, Bruce J.; De Vos, Diana U.S. Pat. Appl. Publ., 15 pp., Cont. of U.S. Ser. No. 366,810, abandoned. CODEN: USXXCO APPLICATION NO. DATE PATENT NO. KIND DATE ______ ____ US 2001-894579 20010628 US 2001046487 20011129 A1 PRAI US 1994-366810 B1 19941230 => d 25-50 au ti so 19 ANSWER 25 OF 50 CAPLUS COPYRIGHT 2003 ACS Langlois, Bruno Drilling fluid containing cellulose nanofibrils and its use for petroleum production PCT Int. Appl., 27 pp. CODEN: PIXXD2 ANSWER 26 OF 50 CAPLUS COPYRIGHT 2003 ACS Hui, Sek Wen; Stoicheva, Natailia; Zhao, Ya-li Method and compositions for high efficiency loading, transfection and fusion of cells by electric pulses U.S., 15 pp. CODEN: USXXAM ANSWER 27 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE Vigano, Alessandra; Bricalli, Dorella; Trabattoni, Daria; Salvaggio, Antonino; Ruzzante, Stefania; Barbi, Maria; Di Sanzo, Giuseppe; Principi, Nicola; Clerici, Mario (1) Immunization with both T cell-dependent and T cell-independent vaccines augments HIV viral load secondarily to stimulation of tumor necrosis factor alpha. AIDS Research and Human Retroviruses, (June 10, 1998) Vol. 14, No. 9, pp. 727-734. ISSN: 0889-2229. ANSWER 28 OF 50 CAPLUS COPYRIGHT 2003 ACS Bronshtein, Victor Loading and unloading of permeating protectants for cell, tissue, and organ cryopreservation by vitrification PCT Int. Appl., 33 pp. CODEN: PIXXD2 ANSWER 29 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE Gouvea, Cibele M. C.; Vidal, Benedito C.; Martins, Ione S. (1)

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ANSWER 47 OF 50 CAPLUS COPYRIGHT 2003 ACS L9 Gamalei, Yu. V. ΑU Phloem loading in woody and herbaceous plants TIFiziologiya Rastenii (Moscow) (1985), 32(5), 866-75, 2 plates SO CODEN: FZRSAV; ISSN: 0015-3303 ANSWER 48 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. L9 SERPERSU E H; KINOSITA K JR; TSONG T Y ΑU REVERSIBLE AND IRREVERSIBLE MODIFICATION OF ERYTHROCYTE MEMBRANE ΤI PERMEABILITY BY ELECTRIC FIELD. BIOCHIM BIOPHYS ACTA, (1985) 812 (3), 779-785. SO CODEN: BBACAQ. ISSN: 0006-3002. ANSWER 49 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. L9 SUBBOTINA YU L; LEVENSON V I; LYUBINSKAYA M M ΑU COMPARATIVE IMMUNOCHEMICAL AND SEROLOGIC INVESTIGATION OF ANTIGENIC TICOMPOSITION OF RIBOSOMES ISOLATED FROM SHIGELLA-FLEXNERI AND SHIGELLA-SONNEI. IMMUNOLOGIYA, (1983) 0 (5), 58-62. SO CODEN: IMMLDW. ANSWER 50 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. L9 WETHERELL J R JR; BLEIWEIS A S AU ANTIGENS OF STREPTOCOCCUS-MUTANS ISOLATION OF A SEROTYPE SPECIFIC AND A ΤI CROSS REACTIVE ANTIGEN FROM WALLS OF STRAIN V-100 SEROTYPE E. INFECT IMMUN, (1978) 19 (1), 160-169. SO CODEN: INFIBR. ISSN: 0019-9567.. => d 26 28 48 bib ab 19 ANSWER 26 OF 50 CAPLUS COPYRIGHT 2003 ACS 1.9 1998:534834 CAPLUS AN 129:119877 DN. Method and compositions for high efficiency loading, transfection and ΤI fusion of cells by electric pulses Hui, Sek Wen; Stoicheva, Natailia; Zhao, Ya-li IN Health Research Inc., USA PA SO U.S., 15 pp. CODEN: USXXAM Patent DT LA English FAN.CNT 1 ALMU DATE APPLICATION NO. DATE PATENT NO. KIND DATE _____ _____ US 1995-439187 19950511 PI US 5789213 A 19980804 PRAI US 1995-439187 19950511 19980804 Methods and compns. are provided for use in electroloading procedures to increase the transfection and fusion efficiency compared to the methods now used in the art. The compns. comprise a two-phase polymer system contg. two water sol. polymers which, when mixed, result in target cells and biol. material being encapsulated into one of the polymer phases in a concd. form. The methods of the present invention for electroloading biol. material into target cells comprises mixing the biol. material into one of the phases of the two-phase polymer system; mixing the target cells into either of the phases of the two-phase polymer system; mixing the phases together to form an emulsion; and exposing the emulsion to a pulsing elec. field in an electroloading process. THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD RE.CNT 7 ALL CITATIONS AVAILABLE IN THE RE FORMAT ANSWER 28 OF 50 CAPLUS COPYRIGHT 2003 ACS L9 1997:803775 CAPLUS AN

128:53192 DN Loading and unloading of permeating protectants for cell, tissue, and ΤI organ cryopreservation by vitrification Bronshtein, Victor IN Universal Preservation Technologies, Inc., USA; Bronshtein, Victor PA PCT Int. Appl., 33 pp. so CODEN: PIXXD2 DTPatent LA English FAN.CNT 1 APPLICATION NO. DATE KIND DATE PATENT NO. ______ _____ WO 1997-US9207 19970529 A1 19971204 WO 9745010 PΙ W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG AU 1997-32900 19970529 A1 19980105 AU 9732900 EP 1997-928712 19970529 19990616 EP 921723 A1 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI JP 1997-542954 19970529 20011002 JP 2001517204 T2Ρ 19960529 PRAI US 1996-18638P W 19970529 WO 1997-US9207 The present invention is directed to a method for cryopreserving a biol. AB sample, including gradually or stepwise loading the sample with permeating protectant by contacting the sample with solns. including the protectant and a non-permeating co-solute that limits the amt. of protectant that penetrates into cells of the biol. specimen. The method further includes the gradual or step of unloading (rehydration) of the sample by contacting the sample with one ore more rehydration solns. having progressively lower concns. of both the protectant and co-solute, such that the protectant is removed from the cells of the sample. Concn. of the co-solute during loading and unloading should be at max. value that still does not damage the sample at room and subzero temps. An example is given for gradual loading and unloading of rat heart with DMSO. ANSWER 48 OF 50 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. Ь9 1985:330630 BIOSIS ANDNBA80:622 REVERSIBLE AND IRREVERSIBLE MODIFICATION OF ERYTHROCYTE MEMBRANE TI PERMEABILITY BY ELECTRIC FIELD. SERPERSU E H; KINOSITA K JR; TSONG T Y ΑU DEP. BIOLOGICAL CHEMISTRY, JOHNS HOPKINS UNIV., SCH. MED., BALTIMORE, MD CS 21205, USA. BIOCHIM BIOPHYS ACTA, (1985) 812 (3), 779-785. so CODEN: BBACAQ. ISSN: 0006-3002. FS BA; OLD LA English Electric fields of a few kV/cm and of duration in .mu.s are known to AB implant pores of limited size in cell membranes. A study of kinetics of pore formation and reversibility of pores is reported. Loading of biologically active molecules was also attempted. For human erythrocytes in an isotonic saline, pores allowed passive Rb+ entry formed within 0.5 .mu.s when a 4 kV/cm electric pulse was used. Pores that admitted oligosaccharides were introduced with an electric pulse of a longer duration in an isosmotic mixture of NaCl and sucrose. These pores were irreversible under most circumstances, but they could be resealed in an osmotically balanced medium. A complete resealing of pores that

admitted Rb+ took .apprx. 40 min at 37.degree. C. Resealing of pores that admitted sucrose took much longer, 20 h, under similar conditions. In other cell types, resealing step may be omitted due to stronger membrane structures. Experimental protocols for loading small molecules into cells without losing cytoplasmic macromolecules are discussed.

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